World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Contribution to modeling the kinetics of phosphatase thermal inactivation

Sobamfou Marius Kambiré [1], Adama Niaré [2], Georges Stéphane Dembélé [3, 5, *], Akassa Marius Bernard Djako [2], Jacques Mankambou Gnanwa [4] and Mamadou Guy-Richard Koné [3, 5]

[1] *General Chemistry Laboratory (LCG), University of Péléforo Gon Coulibaly, BP 1328 Korhogo, Côte d'Ivoire.*
[2] *Fundamental and Applied Physics Laboratory (LPFA), University of Nangui Abrogoua, Abidjan 02, Côte d'Ivoire.*
[3] *Thermodynamics and Environmental Physics Laboratory, University of Nangui Abrogoua, Abidjan 02, Côte d'Ivoire.*
[4] *Agrovalorisation Laboratory, Jean Lorougnon Guédé University, Daloa, BP 150 Daloa, Cote d'Ivoire.*
[5] *Ivorian Research Group in Disease Modeling (GIR2M), Abidjan, Côte-d'Ivoire.*

World Journal of Advanced Research and Reviews, 2024, 24(02), 671–681

Publication history: Received on 24 September 2024; revised on 01 November 2024; accepted on 04 November 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.24.2.3361

Abstract

This article focuses on modeling the kinetics of thermal inactivation of acid phosphatases extracted from breadfruit (*Artocarpus communis L*). By examining the stability of these enzymes at different temperatures, the study highlights their importance in the plant kingdom for fruit preservation, and in the animal kingdom for medical diagnostics. The study demonstrated that the kinetics of thermal inactivation of acid phosphatases P1A, P1B and P2 are influenced by temperature and the substrate used. Analyses revealed that more complex kinetic models, such as two-step models, offer a better representation of inactivation for certain substrates, notably PPNa and pNPP at specific temperatures. This indicates the formation of partially active enzymes in some cases. Understanding these inactivation mechanisms is crucial for optimizing the use of phosphatases in plant applications, such as fruit preservation, and in medical diagnostic contexts. The results obtained provide a solid basis for future research aimed at improving the stability and efficacy of phosphatases in various fields.

Keywords: Acid phosphatases; Kinetic modeling; *Artocarpus communis*; Thermodynamics; Thermal inactivation

1. Introduction

Performing inactivation experiments at several temperatures to obtain information on enzyme stability is quite common [1] . Thus, in this work, we focused on enzymes of a protein nature: acid phosphatases, which are of interest:

- In the plant kingdom, in slowing down fruit degradation. Indeed, once their enzymatic activity has been lowered by isolating and modifying the encoding genes, this could reduce the speed of fruit degradation, thereby prolonging its shelf life ;
- In the animal kingdom, they have diagnostic value. In the case of abnormalities or lesions in a tissue or organ, their concentration increases, which may indicate leukemia, myocardial infarction, pulmonary embolism, bone disease or other metabolic disorders.

In order to make the best use of these phosphatases, it is useful to obtain information on their thermal stability. This information can be obtained from inactivation, which involves subjecting the enzyme to a temperature gradient. The study of thermal inactivation of enzymes can, on the basis of kinetic considerations, reveal the mechanisms by which the enzyme is denatured. Most recently, Gnanwa [2] has described the kinetics of thermal inactivation of three acid phosphatases: P_{1A}, P_{1B} and P_2 extracted from breadfruit and used with the substrates para-nitrophenylphosphate (pNNP), sodium phenylphosphate (PPNa), adenosine-5-triphosphate (ATP) and sodium pyrophosphate (PyPNa) using

* Corresponding author: Dembélé Georges Stéphane

a first-order reaction. In most cases, however, it has been shown that first-order kinetics are not efficient in accounting for thermal inactivation, and more complex models are required. [3]. It is in this context that we propose to reanalyze the results obtained by Gnanwa [2] using other kinetic models in order to obtain the best possible interpretation of thermal inactivation. The general aim of this work will be to describe the kinetics of thermal inactivation of three acid phosphatases : P_{1A}, P_{1B} and P_2 extracted from breadfruit and used with the substrates: pNNP, PPNa, ATP and PyPNa. The specific objectives will then be to determine the kinetic and "thermodynamic" parameters of these phosphatases.

2. Materials and methods

2.1. Material

Two types of material are used in this work. These are purified acid phosphatases extracted from the fruit of the breadfruit tree *Artocarpus communis L* and curveExpert software.

2.2. Methods

2.2.1. Solving non-linear equations: determining kinetic parameters

Rate constants and relative activity of the partially active enzyme

For this study of the thermal inactivation of phosphatases, we had to solve the non-linear equation $a = (1 - \beta^*)e^{-k_1 t}$ for the one-step model $E \overset{k_1}{\rightarrow} E^*$ where the native form of the E enzyme is transformed into a partially active E* form during the inactivation process. For this purpose, the use of software was necessary. The CurveExpert software was of considerable help. We had to calculate the parameters k_1 and β^* (respectively, rate constant and relative activity of the partially active enzyme), neither of which is known in advance. Using the software, we first smoothed the experimental results obtained by Gnanwa [2], then calculated the two parameters by manually initializing the values. It should be recalled that when calculating the relative activity of the partially active enzyme, we were confronted with the problem that some of the values found were not only negative, but also greater than unity (relative activity less than one). For this reason, we often had to manually adjust the initial values of in order to obtain plausible results.

Activation energy

By plotting the logarithms of the relative activities as a function of the inverse of temperature (in Kelvin), we were able to graphically determine the activation energy. From the slope of the curve we calculated the activation energy using the expression :

$$-\frac{E_a}{R} = slope \Rightarrow E_a = -R * slope \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

With R: perfect gas constant ($8.314\ J\ K^{-1}mol^{-1}$)

2.2.2. Determination of "thermodynamic" parameters

The "thermodynamic" parameters, enthalpy variation, free enthalpy variation and entropy variation, were calculated using the methods below.

$$\Delta H^{\#} = E_a - RT : Enthalpy\ variation \ldots\ldots\ldots\ldots\ldots (2)$$

$$\Delta S^{\#} = R\left(lnA - \ln\left(\frac{K_B}{h_P}\right) - lnT\right) : Entropy\ variation \ldots\ldots\ldots\ldots\ldots (3)$$

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} : free\ enthalpy\ variation \ldots\ldots\ldots\ldots (4)$$

K_B is Boltzmann's constant (1.38 x 10^{-23} J/K), h_P Planck's constant (6.626 x 10^{-34} J.s) and T the absolute temperature (in Kelvin).

A: Arrhenius constant (in s^{-1})..

3. Results and discussion

The kinetic study of thermal inactivation of the three phosphatases: P_{1A}, P_{1B} and P_2 in the presence of substrates was carried out by considering two single-step mechanisms $E \overset{k_1}{\rightarrow} D$ (model 1) and $E \overset{k_1}{\rightarrow} E^*$ (model 2), which leads respectively to the following relative activities :

$$a = e^{-k_1 t} \; and \; a = (1 - \beta^*)e^{-k_1 t} + \beta^*$$

The results obtained in each case are presented below. Then, with reference to the coefficients of determination, we selected the model that best describes the phosphatase thermal inactivation process.

3.1. The case of phosphatase P_{1A}

3.1.1. pNPP as substrate

The results obtained are shown in Table 1. From 55 to 70 °C, the coefficient of determination is virtually the same for both models. At 75 °C, the coefficient obtained by model 1 is significantly higher than that of model 2. We can therefore conclude that model 1 best represents thermal inactivation, i.e. when pNPP is used as a substrate there would be inactivation of the enzyme.

Table 1 Kinetic parameters of P_{1A} phosphatase when pNPP is used as substrate

	Temperature (°C)	$E \overset{k_1}{\rightarrow} D: a = e^{-k_1 t}$		$E \overset{k_1}{\rightarrow} E^*: a = (1 - \beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
pNPP	**55**	0.0134	0.9897	0.01397	0.0313208	0.9891
	60	0.0211	0.9622	0.0237	0.06748952	0.9650
	65	0.0293	0.9716	0.0349	0.08530075	0.9775
	70	0.0417	0.9693	0.0584	0.12768244	0.9720
	75	0.0677	0.9726	0.1128	0.16349269	0.8879

3.1.2. ATP as a substrate

The results obtained are presented in Table 2. From 45 to 70 °C, the coefficient of determination is practically the same for both models. We therefore conclude that model 1 best represents the process of thermal inactivation, i.e. when ATP is used as a substrate, the enzyme is inactivated.

Table 2 Kinetic parameters of P_{1A} phosphatase when ATP is used as substrate

	Température (°C)	$E \overset{k_1}{\rightarrow} D: a = e^{-k_1 t}$		$E \overset{k_1}{\rightarrow} E^*: a = (1 - \beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
ATP	**45**	0.0090	0.9861	0.009033	0.0012105	0.9861
	50	0.0135	0.9962	0.01361	0.00270633	0.9961
	55	0.0223	0.9958	0.02259	0.00832802	0.9956
	60	0.0294	0.9882	0.03012	0.0133823	0.9884
	65	0.0464	0.9898	0.04876	0.0203806	0.9902
	70	0.0640	0.9932	0.06967	0.0297876	0.9949

3.1.3. PPNa as a substrate

The results obtained are shown in Table 3. At 50 °C and from 60 to 75 °C, the coefficient of determination is virtually the same for both models. However, at 55 °C, the coefficient obtained by model 2 is significantly higher than that of

model 1. Model 2 therefore best represents thermal inactivation, i.e. when PPNa is used as a substrate, a partially active enzyme would be formed during the thermal inactivation process.

Table 3 Kinetic parameters of P_{1A} phosphatase when PPNa is used as substrate

	Temperature (°C)	$E \xrightarrow{k_1} D: a = e^{-k_1 t}$		$E \xrightarrow{k_1} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
PPNa	**50**	0.0052	0.9897	0.005239	0.00405396	0.9897
	55	0.0089	0.9325	0.008959	0.00656091	0.9932
	60	0.0136	0.9951	0.01380	0.00797495	0.9950
	65	0.0179	0.9923	0.01817	0.00969375	0.9923
	70	0.0269	0.9939	0.02750	0.01225204	0.9939
	75	0.0483	0.9985	0.04984	0.01307564	0.9975

3.1.4. PyPNa as a substrate

The results obtained are presented in Table 4. From 40 to 65 °C, the coefficient of determination is practically the same for both models. But at 70 °C, the coefficient obtained by model 1 is significantly higher than that of model 2. We therefore conclude that model 1 best represents thermal inactivation, i.e. when PyPNa is used as a substrate, the enzyme is inactivated.

Table 4 Kinetic parameters of P_{1A} phosphatase when PyPNa is used as substrate

	Temperature (°C)	$E \xrightarrow{k_1} D: a = e^{-k_1 t}$		$E \xrightarrow{k_1} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
PyPNa	**40**	0.0059	0.9946	0.006074	0.01997054	0.9948
	45	0.0095	0.9955	0.01001	0.04138487	0.9953
	50	0.0122	0.9986	0.01329	0.06330506	0.9983
	55	0.0156	0.9949	0.01794	0.09113066	0.9960
	60	0.0213	0.9876	0.02604	0.11271013	0.9879
	65	0.0273	0.9843	0.04521	0.21980797	0.9832
	70	0.0447	0.9891	0.09240	0.263728	0.8450

3.2. The case of phosphatase P_{1B}

3.2.1. pNPP as a substrate

Table 5 Kinetic parameters of phosphatase P_{1B} when pNPP is used as substrate

	Temperature (°C)	$E \xrightarrow{k_1} D: a = e^{-k_1 t}$		$E \xrightarrow{k_1} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
pNPP	**55**	0.0117	0.9609	0.0123	0.03737748	0.9961
	60	0.0168	0.9940	0.01849	0.06112441	0.9948
	65	0.0223	0.9837	0.02551	0.07654764	0.9858
	70	0.0288	0.9768	0.03757	0.12513889	0.9837
	75	0.0377	0.9750	0.05255	0.13297302	0.9765

The results obtained are presented in Table 5. From 60 to 75 °C, both models have practically the same coefficients of determination. However, at 55 °C, the coefficient obtained by model 2 is significantly higher than that of model 1. Model 2 therefore best represents thermal inactivation, i.e. when pNPP is used as a substrate, a partially active enzyme would be formed during the thermal inactivation process.

3.2.2. ATP as a substrate

The results obtained are presented in Table 6. From 50 to 70 °C, the two models have practically the same coefficients of determination. However, at 75 °C, the coefficient obtained by model 1 is significantly higher than that of model 2. Model 1 therefore best represents thermal inactivation, i.e. when ATP is used as a substrate, enzyme inactivation would occur.

Table 6 Kinetic parameters of phosphatase P_{1B} when ATP is used as substrate

	Temperature (°C)	$E \overset{k_1}{\rightarrow} D: a = e^{-k_1 t}$		$E \overset{k_1}{\rightarrow} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
ATP	**50**	0.0106	0.9952	0.01075	0.01344384	0.9951
	55	0.0155	0.9925	0.0160	0.02092447	0.9925
	60	0.0197	0.9927	0.0245	0.1260318	0.9946
	65	0.0271	0.9441	0.03580	0.13104874	0.9585
	70	0.0432	0.9674	0.06473	0.15121434	0.9505
	75	0.0735	0.9861	0.12799	0.18380461	0.8142

3.2.3. PPNa as a substrate

The results obtained are presented in Table 7. When PPNa is used as a substrate, in the temperature range 50-80 °C, the coefficients of determination of both models are essentially the same. Thus, both models best describe thermal inactivation.

Table 7 Kinetic parameters of phosphatase P_{1B} when PPNa is used as substrate

	Temperature (°C)	$E \overset{k_1}{\rightarrow} D: a = e^{-k_1 t}$		$E \overset{k_1}{\rightarrow} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
PPNa	**50**	0.0060	0.9789	0.006129	0.00663419	0.9825
	55	0.0091	0.9907	0.009196	0.00849485	0.9905
	60	0.0114	0.9727	0.01159	0.01131035	0.9723
	65	0.0177	0.9934	0.01805	0.01429528	0.9930
	70	0.0253	0.9955	0.02622	0.01979092	0.9949
	75	0.0356	0.9987	0.03744	0.02374536	0.9981
	80	0.0522	0.9949	0.05623	0.02886308	0.9956

3.2.4. PyPNa as a substrate

The results obtained are shown in Table 8. From 50 to 80 °C, both models show practically the same coefficients of determination. Thus, both models best describe thermal inactivation.

Table 8 Kinetic parameters of phosphatase P_{1B} when PyPNa is used as substrate

	Temperature (°C)	$E \xrightarrow{k_1} D: a = e^{-k_1 t}$		$E \xrightarrow{k_1} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
PyPNa	40	0.0067	0.9780	0.006669	0.0009689	0.9779
	45	0.0107	0.9874	0.01067	0.00108927	0.9874
	50	0.0141	0.9856	0.01419	0.00257097	0.9855
	55	0.0195	0.9942	0.01966	0.00365319	0.9942
	60	0.0272	0.9898	0.02743	0.00480111	0.9895
	65	0.0373	0.9963	0.03787	0.00718655	0.9960
	70	0.0516	0.9906	0.05297	0.01048087	0.9908

3.3. The case of phosphatase P_2

The results obtained are presented in Tables 9, 10, 11 and 12. For the four substrates used, the two models best describe the thermal inactivation process.

3.3.1. pNPP as a substrate

Table 9 Kinetic parameters of phosphatase P_2 when pNPP is used as substrate

	Temperature (°C)	$E \xrightarrow{k_1} D: a = e^{-k_1 t}$		$E \xrightarrow{k_1} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
pNPP	55	0.0104	0.9872	0.01076	0.00128067	0.9884
	60	0.0177	0.9937	0.01796	0.00809069	0.9938
	65	0.0233	0.9946	0.02413	0.0210081	0.9953
	70	0.0334	0.9938	0.03776	0.058216	0.9928
	75	0.0467	0.9825	0.06344	0.11402677	0.9773

3.3.2. ATP is used as a substrate

Table 10 Kinetic parameters of phosphatase P_2 when ATP is used as substrate

	Temperature (°C)	$E \xrightarrow{k_1} D: a = e^{-k_1 t}$		$E \xrightarrow{k_1} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
ATP	50	0.0089	0.9888	0.008942	0.00259969	0.9887
	55	0.0149	0.9944	0.01493	0.00296095	0.9944
	60	0.0220	0.9928	0.02227	0.00755609	0.9925
	65	0.0296	0.9929	0.03037	0.01271553	0.9931
	70	0.0398	0.9919	0.04247	0.02812164	0.9927
	75	0.0531	0.9907	0.06043	0.04920741	0.9891

3.3.3. PPNa as a substrate

Table 11 Kinetic parameters of phosphatase P_2 when PPNa is used as substrate

	Temperature (°C)	$E \overset{k_1}{\rightarrow} D: a = e^{-k_1 t}$		$E \overset{k_1}{\rightarrow} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	β^*	r^2
PPNa	50	0.0073	0.9823	0.007316	0.0015433	0.9823
	55	0.0129	0.9901	0.01320	0.00225564	0.9898
	60	0.0202	0.9987	0.02031	0.0025469	0.9987
	65	0.0307	0.9953	0.03091	0.00305579	0.9952
	70	0.0461	0.9989	0.04675	0.00560833	0.9987
	75	0.0692	0.9887	0.07067	0.00693637	0.9897

3.3.4. PyPNa as substrate

Table 12 Kinetic parameters of phosphatase P_2 when PyPNa is used as substrate

	Temperature (°C)	$E \overset{k_1}{\rightarrow} D: a = e^{-k_1 t}$		$E \overset{k_1}{\rightarrow} E^*: a = (1-\beta^*)e^{-k_1 t} + \beta^*$		
		k_1	r^2	k_1	r^2	k_1
PyPNa	40	0.0039	0.9820	0.003943	0.00861877	0.9819
	45	0.0070	0.9854	0.007091	0.01007525	0.9853
	50	0.0099	0.9944	0.010050	0.011084	0.9943
	55	0.0133	0.9942	0.01356	0.0118805	0.9940
	60	0.0199	0.9967	0.02030	0.01284518	0.9964
	65	0.0276	0.9978	0.02841	0.0148862	0.9974
	70	0.0394	0.9969	0.04100	0.01817321	0.9966

At the end of this analysis, we have selected the best models capable of best describing the phenomenon of thermal inactivation of these phosphatases. However, it should be pointed out that, when model 2 is the best, we retain it for the calculation of kinetic and thermodynamic parameters, and if it's model 1, we recall that it has already been studied with these same phosphatases by Gnanwa [2]. Furthermore, when neither of the two models is superior, we retain the simpler model, i.e. model 1, as the best, since the reduction in the number of parameters reduces the risk of cross-correlation and thus enables better estimation of the apparent rate constants (which are, in many cases, the relevant information required).

3.4. Determination of kinetic and "thermodynamic" parameters

We have calculated the kinetic and "thermodynamic" parameters in cases where model 2 is better.

3.4.1. Kinetic parameters

Phosphatase activation energy

The graphical representations of Ln k as a function of the inverse of temperature (in Kelvin) all describe affine lines with negative slopes (Figures 1 and 2). The equations of these lines follow Arrhenius' law, with slopes of $(-\frac{E_a}{R})$ were used to calculate phosphatase activation energies (Table 13).

- **P_{1A} phosphatase with PPNa as substrate**

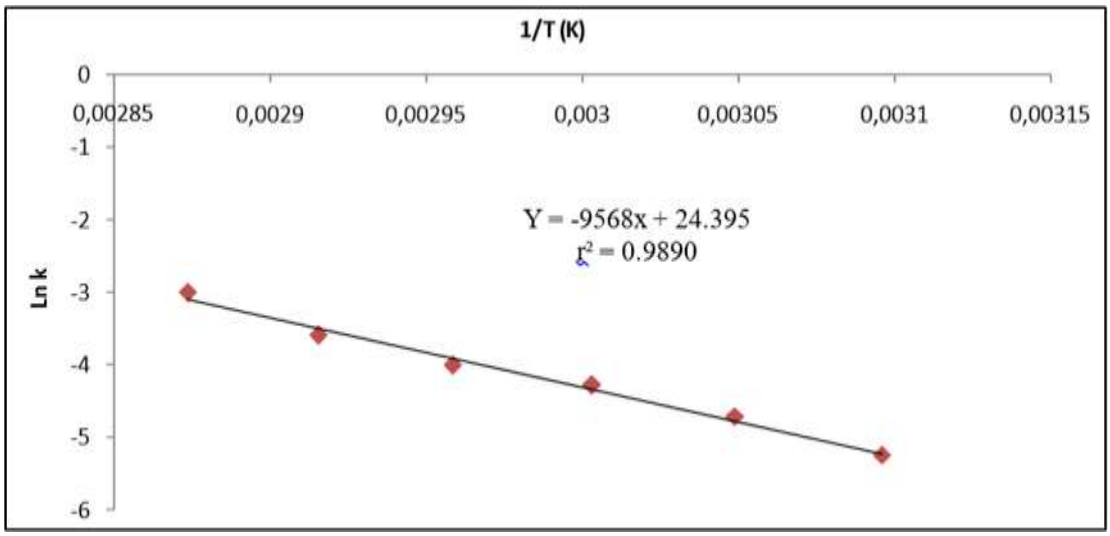

Figure 1 Ln k as a function of the inverse of the temperature of P_{1A} phosphatase inactivation, with PPNa used as substrate

- **P_{1B} phosphatase with pNPP as substrates**

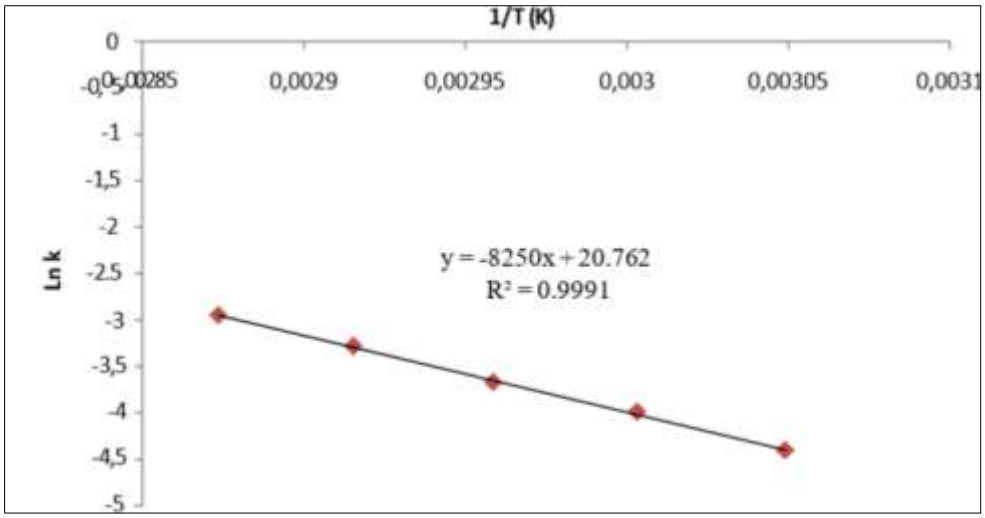

Figure 2 Ln k as a function of the inverse of the temperature of P_{1B} phosphatase inactivation, with pNPP used as substrate

Activation energy is the energy required to initiate the thermal inactivation process. The high, positive activation energy values (Table 13) indicate that these phosphatases do not inactivate as quickly. For the substrates, the very high values suggest that the inactivation process involves the simultaneous breaking of a multitude of bonds, and that the energy barrier is greater than the simple reaction [2]. Hence the need for a high energy input to the enzyme-substrate complex to initiate inactivation, probably due to a possible compact structure of the enzymes and the thiol (SH) group or disulfide bridges at the active sites [4]. These activation energies are significantly higher on average than those obtained by Gnanwa et *al.*[2]. What's more, the values we found are much lower than those obtained by Alina et *al.* [5] in their work on alkaline phosphatases in raw bovine and caprine milk. These values are 421 and 406 kJ/mol respectively.

Table 13 Activation energy of P_{1A} and P_{1B} phosphatases when PPNa and pNPP are used as substrates respectively

Enzyme type	Substrate type	Activation energy (kJ/mol)
P_{1A}	PPNa	79.548
P_{1B}	pNPP	68.590

Apparent activation energy of partially active enzymes

- **P_{1A} phosphatase when PPNa is used as a substrate**

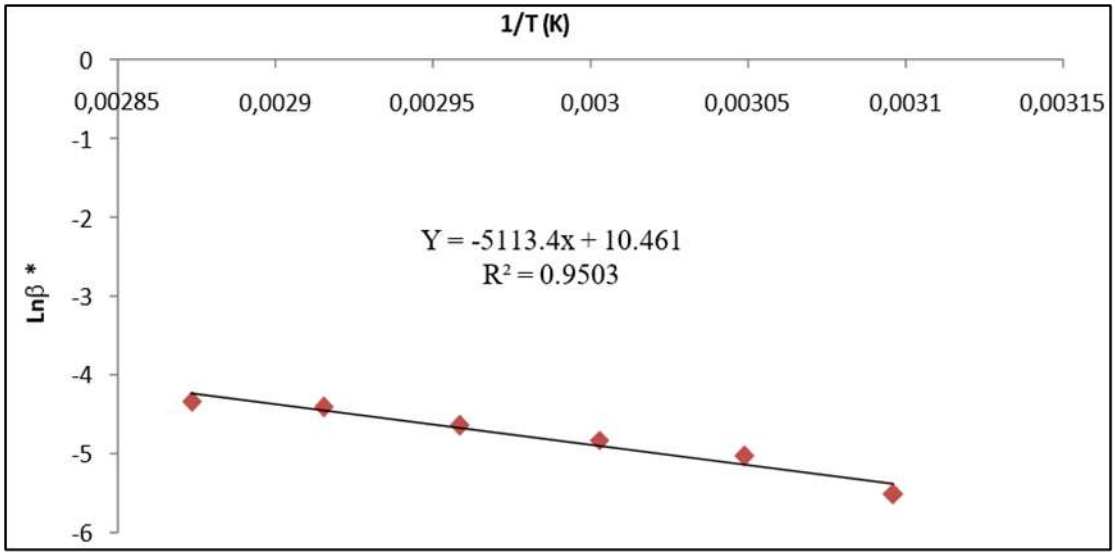

Figure 3 Ln β^* as a function of inverse temperature of P1A phosphatase inactivation with PPNa as substrate

- **P_{1B} phosphatase when pNPP is used as substrate**

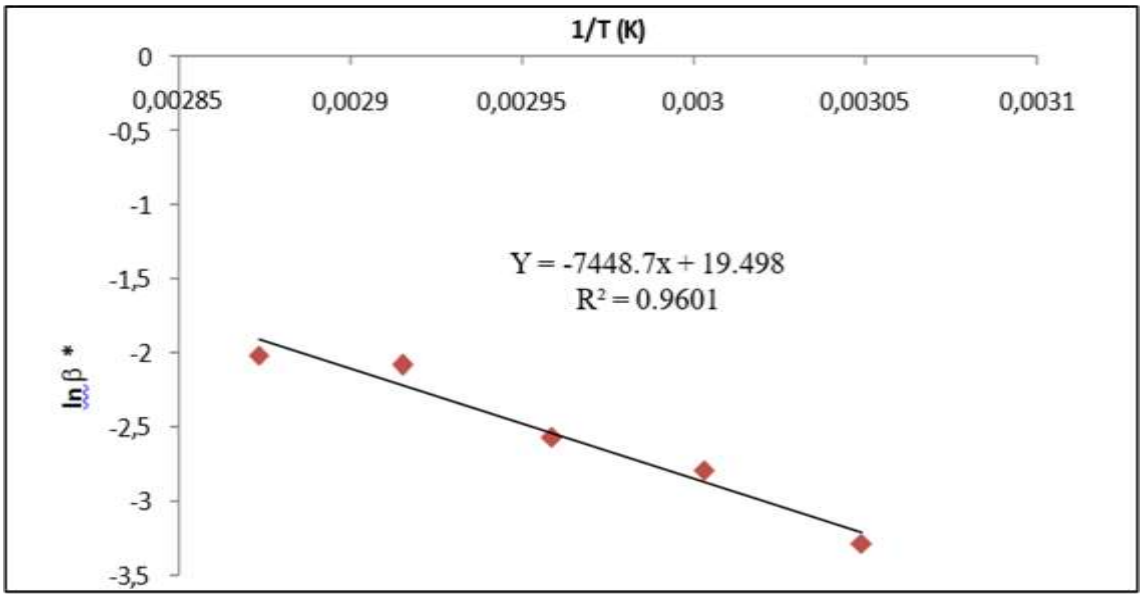

Figure 4 Ln β^* as a function of inverse temperature of P_{1B} phosphatase inactivation, with pNPP used as substrate

The apparent activation energy of the partially active species was calculated from the curve Ln β^* as a function of inverse temperature. This energy is particularly low compared with that of native phosphatases, especially when P_{1A} is

used with phenylphosphate as a substrate, implying that in the presence of this substrate, the process of inactivation of the E* species (of these two phosphatases) is not rapidly initiated.

Table 14 Apparent activation energy of partially active enzymes during inactivation between 40 and 80 °C when PPNa and pNPP are used as substrates respectively

Enzyme type	Substrate type	Activation energy (kJ/mol)
P_{1A}	PPNa	42.513
P_{1B}	pNPP	61.928

3.4.2. Thermodynamic parameters

Tables 15 and 16 show the values of the various thermodynamic parameters of the P_{1A} and P_{1B} phosphatase inactivation process, providing Model 2 with a better description of the thermal inactivation mechanism. The enthalpy ($\Delta H^{\#}$) and free enthalpy ($\Delta G^{\#}$) values are all positive and high. These results show that the inactivation process of these two phosphatases in the presence of PPNa and pNPP respectively is not only endothermic and non-spontaneous, but that there are weak molecular interactions between the phosphatases and the substrates. Furthermore, the entropy values found are all negative. These negative entropy values would be due to the fact that the structural disorder states of these biocatalysts occurred during the transition stage following thermal inactivation, hence the increase in enthalpy.

P1A phosphatase with PPNa as substrate

Table 15 shows the thermodynamic parameters of phosphatase P_{1B} used with pNPP as substrate. The enthalpy and entropy values found are all lower than those obtained by Gnanwa [2] with model 1. On the other hand, our free enthalpy values are all roughly equal to those of Gnanwa [2].

Table 15 Thermodynamic parameters for thermal inactivation of P_{1A} phosphatase between 50 and 75°C in the presence of PPNa

Temperature (°C)	Thermodynamic parameters		
	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J mol^{-1} K^{-1})	$\Delta G^{\#}$ (kJ/mol)
	Phénylphosphate		
50	76.862	-42.752	90.671
55	76.821	-42.880	90.886
60	76.779	-43.005	91.100
65	76.738	-43.129	91.316
70	76.696	-43.251	91.531
75	76.655	-43.372	91.748

La phosphatase P_{1B} avec le pNPP utilisé comme substrat

Table 16 shows the thermodynamic parameters of phosphatase P_{1B} used with pNPP as substrate. The enthalpy and entropy values are all higher than those obtained by Gnanwa [2] with model 1. On the other hand, our free enthalpy values are all approximately equal to those of Gnanwa [2].

Table 16 Thermodynamic parameters for thermal inactivation of phosphatase P1B between 55 and 75 °C in the presence of pNPP

Temperature (°C)	Thermodynamic parameters		
	$\Delta H^{\#}$ (kJ/mol)	$\Delta S^{\#}$ (J mol^{-1} K^{-1})	$\Delta G^{\#}$ (kJ/mol)
	pNPP		
50	65.863	-73.084	89.834
55	65.821	-73.210	90.200
60	65.780	-73.334	90.567
65	65.738	-73.456	90.933
70	65.697	-73.576	91.301
75	65.863	-73.084	89.834

4. Conclusion

In this work, we analyzed the thermal inactivation of the three phosphatases P_{1A}, P_{1B} and P_2 using two kinetic models based on the following mechanisms: $E \xrightarrow{k_1} D$ (model 1) and $E \xrightarrow{k_1} E^*$ (model 2). The following results were obtained:

- For the P_{1A} enzyme, model 1 best represents the mechanism of thermal inactivation when pNPP, ATP and PyPNa are used as substrates, whereas with PPNa model 2 prevails.
- For the P_{1B} enzyme, model 2 best describes the thermal inactivation process when the pNPP substrate is used. On the other hand, with ATP, model 1 is better, and with PPNa and PyPNa, both models prevail.
- For the P_2 enzyme, both models perfectly account for the phenomenon of thermal inactivation.

Thermodynamic quantities were calculated on the basis of the data obtained from the models. Thus, for the two phosphatases P_{1A} and P_{1B}, offering a better description of the phenomenon of thermal inactivation when the substrates PPNa and pNPP are used respectively, the enthalpy ($\Delta H^{\#}$) and free enthalpy ($\Delta G^{\#}$) values are all positive and high. On the other hand, the entropy values found are all negative. This work, which is a contribution to the modeling of phosphatase thermal inactivation kinetics, should be continued. Indeed, the curve Expert software has shown some limitations in non-linear regression, so it is important to build a more powerful program. This will enable more complex models to be analyzed.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] G. Greco, D. Pirozzi et L. Gianfreda, «Thermal equivalence criteria in the chemical deactivation and stabilization of acid phosphatase,» Enzyme and Microbial Technology, vol. 13, pp. 353- 358, 1991.

[2] M. J. Gnanwa, K. H. Konan, N. S. Gnangui, N. E. P. Kouadio et L. Kouamé, «Study of breadfruit (Artocarpus communis) seeds: kinetic and thermodynamique analysis,» Journal of Animal & Plant Sciences, vol. 21, n°11, pp. 3233-3240, 2014.

[3] V. Peter, P. Milan, S. Vladimir et B. Vladimir, «Analysis of mechanism and kinetics of thermal inactivation of enzymes: Evaluation of multitemperature data applied to inactivation of yeast invertase,» Enzyme and Microbial Technology, vol. 20, pp. 346-354, 1997.

[4] M. Ladero, R. Ferrero, A. Vian, A. Santos et F. Garcia-Ochoa, «Kinetic modelling of the thermal and pH inactivation of a thermostable b-galactosidase from Thermus sp. strain T2,» Enzyme and Microbial Technology, vol. 37, pp. 505-513, 2005.

[5] W. Alina, B. Jolanta, I. Viera et P. Milan, «Kinetics of thermal inactivation of alkaline phosphatase in bovine and caprine milk and buffer,» International Dairy Journal , vol. 17, pp. 579-586, 2006.